Homogeneity and Evaluation of the New NIST Leaf Certified Reference Materials

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ABSTRACT

The NIST has produced and is in the process of certifying two new leaf CRMs, SRM1515 Apple Leaves and SRM 1547 Peach Leaves, as replacements for the no longer available NBS Orchard Leaves and the almost depleted Citrus Leaves. These two new materials have been processed and are being thoroughly evaluated and should provide the most advanced natural matrix botanical trace-element reference materials available. Caution should be used in determining a basis weight (drying) for these CRMs because of their very fine particle size. Homogeneity has been established by instrumental neutron activation analysis on both leaf materials for five elements, to date, to better than 1.5% (1 *s*) for 100-mg sample sizes.

Index Entries: Certified reference materials; standard reference materials; trace-element analysis; neutron activation analysis; homogeneity; botanical materials.

INTRODUCTION

The first natural matrix, widely distributed reference material was Bowen's Kale, produced and evaluated by Humphrey J.M. Bowen of Reading University, UK (*1*). This material demonstrated to the scientific community the usefulness of such reference materials, and shortly afterwards the National Bureau of Standards (NBS) brought forth the first such material to be certified for trace-elemental content (*2*). Eventually, SRM 1571, Orchard Leaves, was certified by NBS for 25 elements, with

information values and/or compiled literature values for 42 additional elements (3), for a total of 67 elements. The Orchard Leaves SRM has been out of stock for a number of years and was replaced by SRM 1572, Citrus Leaves. The Citrus Leaves SRM was certified by NBS for 22 elements, with information values and/or compiled literature values for 35 additional elements, for a total of 57 elements.

The National Institute of Standards and Technology (NIST; formerly NBS) has obtained and prepared a new orchard leaves reference material, to be certified for elemental content. Actually, the previous orchard leaves are being replaced by two new leaf materials: Apple Leaves, SRM 1515; and Peach Leaves, SRM 1547. These two materials have been obtained and prepared for certification work, and this paper discusses some of the work on these materials to date.

Since the intent was to improve these new leaf SRMs in relation to both the old leaf SRMs and other currently available botanical reference materials, a number of new or revised procedures were developed to better evaluate the SRM preparation procedures. These included: hand-picking the apple leaves followed, prior to grinding, by washing, to help remove dirt and siliceous material (a significant problem with Orchard Leaves; washing was not done with the peach leaves, to determine differences, if any); jet-milling and air-classification to obtain very small particle size, thus allowing evaluation of homogeneity at smaller sample sizes; a detailed moisture-content and moisture-uptake evaluation, along with weight-loss measurements during drying; and a dissolution study both to help establish the "insolubles" content of the leaves, if any, and to evaluate the ability of different dissolution techniques to totally solubilize the leaf materials.

PROCESSING

The apple leaves were collected and initially processed by Cyril B. Smith of the Department of Plant Nutrition, Pennsylvania State University. Leaves were hand-picked from the midshoot portions of trees that were free from trace-element sprays and pesticides. Leaves were placed into approximately 650 paper grocery bags and refrigerated until they could be washed and ground. They were washed in a dilute solution of a nonionic, phosphorus-free detergent, dipped in tap water, and then rinsed three times in distilled water. They were dried, in cardboard trays with clean paper liners, in an oven at 65°C. They were then ground in a stainless steel Wiley mill to pass a 40-mesh screen. The processing was in batches, with emission spectroscopic analysis of each batch showing no detectable differences between batches.

The peach leaves were obtained through R. A. Isaac at the Soil Testing and Plant Analysis Laboratory of the College of Agriculture, University of Georgia. They provided approximately 600 pounds of dried leaves, including preliminary grinding and sieving.

At NIST, the leaves were further ground against themselves in a jet-mill and air-classified to pass 200 mesh. They were thoroughly blended in a large V-blender, and then radiation-sterilized in bulk through exposure to cobalt-60 gamma radiation. The apple and peach leaves were each bottled in approximately 5000 clean amber glass bottles, with 50 g of leaves/bottle.

DRYING STUDY

The finely ground leaf materials were evaluated for weight loss during drying and moisture pickup by J. Moody and T. Vetter of the NIST Inorganic Analytical Research Division. The drying techniques evaluated were: convection oven at 85°C, vacuum oven at room temperature with liquid nitrogen cold trap, desiccator drying using anhydrous magnesium perchlorate desiccant, and freeze-drying. The space available here is not sufficient to present these data; however, only desiccator-drying and true freeze-drying (with the sample frozen prior to application of vacuum and kept at −5°C during the entire process) provided acceptable weight-loss results. The conventional oven and vacuum oven techniques were not acceptable. Under the two recommended drying conditions, weight losses were approximately 2.0% for the apple leaves and 2.3% for the peach leaves.

Samples of both apple leaves and peach leaves from bottles used for the homogeneity analyses (15 bottles for each leaf SRM) were again checked for weight losses, using the approved freeze-drying technique. These data provided confirmatory values of 1.68 ± 0.19% (1 *s*) weight loss for the apple leaves, and 2.08 ± 0.11% (1 *s*) weight loss for the peach leaves.

It is believed that these materials are very sensitive to the drying technique because of their extremely fine particle size. Even very gentle drying techniques that had been used successfully for other conventionally ground botanical materials gave substantially higher weight losses for these jet-milled materials, due most likely because of release and loss of the natural volatile oils in the leaves. Thus, for actual analyses of these materials, it may be preferable that undried samples be used, with weight-loss corrections determined on separate samples and applied to the final calculations.

A moisture-uptake study was also made on these materials, prior to the homogeneity study, to help evaluate appropriate sample-handling protocols. In our laboratory atmosphere (21°C), the undried apple leaves picked up 0.15% by weight in 4.0 min, whereas the dried apple leaves picked up 1.12% in 4.0 min. For the peach leaves, the weight gain was 0.23% for the undried material and 0.88% for the dried leaf material. These data indicate that analytical uncertainties can be significantly reduced by working with the undried leaf material, and thus, for the

homogeneity study, undried samples were taken for analysis and the appropriate correction for weight loss upon drying made in the final calculations. In addition, sample weighing was completed as quickly as possible after opening the sample bottles.

DISSOLUTION STUDY

In order to better understand and eliminate some of the dissolution problems that had been found for Orchard Leaves, dissolution studies are being performed on both leaf materials. In these studies, scientists from a number of different analytical groups at NIST are applying their chosen dissolution methods to leaf samples that have been irradiated in the NBS Reactor. After dissolution is complete, each solution is filtered, and the filters and dissolution containers are counted on a germanium detector and gamma-ray analysis system to measure any remaining or insoluble residue. This dissolution evaluation is being coordinated by R. Greenberg, and the results will be reported in detail elsewhere (*4*). However, it can be stated that to date many of the dissolution techniques showed, on the filter paper, one or more elements present above the percent level (relative to the total amount contained in the material).

EXPERIMENTAL

Since these leaf materials were ground to extremely fine particle sizes through use of the jet-mill, it was expected that a very high degree of homogeneity would be obtained. The homogeneity study was thus designed to establish whether a sample size smaller than the usual 250 mg could be specified, while still maintaining appropriate certification uncertainties. The homogeneity study was made by INAA with a sample size of 100 mg.

Experimental conditions were as follows: Short-half-life isotopes were measured after a 60-s irradiation at 2×10^{13} n · $cm^{-2}s^{-1}$ fluence rate, with four counts, usually at 2, 9, 22, and 60 min decay. Counting geometries were always 10 cm for these counts, and data were corrected for differences in irradiation time (using copper-foil fluence monitors), decay time before counting, live time of count, decay during counting period, pulse pileup, and weight losses during drying. For these preliminary results, the elements determined under these conditions were Al, Ca, Mn, and K.

Activation products with half-lives of 1 d or more were determined after a second irradiation on these same samples, this time for 4 h at a fluence rate of 6×10^{13} n·$cm^{-2}s^{-1}$. Samples were counted twice, after approximately 6 and 30 d. These 4-h irradiations were actually two irradiations of 2 h each. After the first irradiation, each rabbit was immediately flipped end-for-end and irradiated for an additional 2 h. With the

specific configuration of the NBSR pneumatic tubes, this exactly corrects for the linear fluence dropoff in these tubes, providing all samples with exactly the same neutron exposure (within measurement uncertainties of $\pm 0.25\%$). For these preliminary results, the element lanthanum was measured under these conditions.

In addition to those corrections specified above, conditions were monitored as closely as possible to eliminate or minimize all sources of error in these analyses.

RESULTS

This study is still in process, and the elements evaluated for homogeneity to date are Al, Mn, K, Ca, and La for both materials. The results for SRM 1515, Apple Leaves, are contained in Table 1, and results for SRM 1547 are contained in Table 2. Evaluation of the data shows that for Apple Leaves SRM, three of the five elements measured have observed variations that fall *within* the 5–95% range of expected variances for a normal population described by the given counting statistics (σ). For Peach Leaves SRM, four of the five elements have observed experimental uncertainties that fall *outside* of that range, suggesting that washing of Apple Leaves may have slightly improved the observed sample variability. However, the measured homogeneity of both these materials is still very good, with "Additional Variance" requirements of, at most, not much over 1%. It should be noted that the "Additional Variance" value includes all experimental uncertainties in the entire measurement process (preparation, irradiation, counting, and data reduction), except for the counting statistics (σ).

In order to evaluate the effect of the copper-foil fluence monitor correction on the short half-life measurements, potassium values shown in Table 1 for Apple Leaves were recalculated without using the fluence monitor correction factor. The copper-foil data had an experimentally observed standard deviation of 1.014% (1 s) for 42 irradiations ($n = 42$) over a 56-d period, whereas the counting statistics for these data (σ) averaged 0.11%. Without the copper-foil fluence correction factor, the Apple Leaf potassium values had SD = 1.07%, $\sigma = 0.56\%$ (since counting statistics are the same), and an "Additional Variance" of 1.00%. Since use of the fluence normalization factors reduced the experimentally observed variability (s) from 1.07 to 0.55%, this demonstrates the need for such fluence monitoring when high-precision data are required and when irradiations are performed over a relatively long period of time. Further, the above data were calculated with only 13 data points for the non-fluence-corrected potassium values, because one irradiation was known to be about 12% longer than the others as a result of operator error. However, when the fluence monitor correction was applied, this value was indistinguishable from the other 13 values and, thus, was included in the fluence-corrected data in Table 1.

Table 1
Homogeneity Results for SRM 1515, Apple Leaves

Element	n	s[a]	σ[b]	Excess Variance[c] Observed?	Additional[d] Variance
Potassium	14[e]	0.55%	0.56%	No	0.0%
Manganese	14	0.63%	0.16%	Yes	0.62%
Aluminum	15	0.72%	0.61%	No	0.42%
Calcium	13[f]	0.92%	0.91%	No	0.30%
Lanthanum	15	1.07%	0.26%	Yes	1.07%

[a]s about unweighted mean (in % relative).
[b]*A priori* uncertainty based only on counting statistics (in % relative).
[c]Determination of Excess Variance based upon agreement between s and σ (i.e., whether s fell between 5–95% probability for a normal population).
[d]Additional variance required to bring "probability of exceeding" to 50 ± 2% for a normal population.
[e]One homogeneity sample was lost for all determinations except aluminum.
[f]A second sample was counted at a different geometry, thus could not be directly compared to the others.

Table 2
Homogeneity Results for SRM 1547, Peach Leaves

Element	n	s[a]	σ[b]	Excess Variance[c] Observed?	Additional[d] Variance
Potassium	15	1.03%	0.45%	Yes	0.95%
Manganese	15	0.74%	0.10%	Yes	0.76%
Aluminum	15	1.44%	0.61%	Yes	1.35%
Calcium	15	0.89%	0.91%	No	0.0%
Lanthanum	15	0.79%	0.17%	Yes	0.74%

[a]s about unweighted mean (in % relative).
[b]*A priori* uncertainty based only on counting statistics (% relative).
[c]Determination of Excess Variance based upon agreement between s and σ (i.e., whether s fell between 5–95% probability for a normal population).
[d]Additional variance required to bring "probability of exceeding" to 50 ± 2% for a normal population.

In conclusion, the data in Tables 1 and 2 indicate that these two new SRMs are homogeneous at the 100-mg sample size to better than ±1.5% at the 1 *s* confidence level for the five elements evaluated. Further homogeneity analyses on seven additional elements in these materials are reported elsewhere (*5*).

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